30.5 CMOS Integrated DNA Chip for Quantitative DNA Analysis

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DNA chip technology has become a standard means of research in both DNA and RNA analyses. At present, fluorescence detection is the most widely used method [1]. However, it requires a complicated process of labeling the target DNA with fluorescent dye, costly arrays, and costly equipment for fluorescence analysis. Extensive efforts have been made to solve the above problems. Among various new technologies, the electrochemical DNA detection method is promising because it dispenses with the costly optical system and can utilize semiconductor technology. Recently, DNA sensors utilizing CMOS circuit technology were reported. Although Thewes et al. [2] succeeded in constructing a fully electronic DNA sensor, they still need complicated DNA labeling with enzymes, which are indispensable for the electrochemical signals in their method.

This paper presents an electrochemical DNA detection method that dispenses with complicated labeling. Figure 30.5.1 shows the principle of our electrochemical DNA detection method [3]. DNA probes are immobilized on Au electrodes. A solution of the target DNA is then applied to the probe DNA, and excess solution is washed away using buffer solutions. After these hybridization and washing processes, double-stranded DNA is formed only when the target DNA and probe DNA are completely matched. Then a solution of intercalators is added, which specifically bind with the double-stranded DNA molecules. When a voltage is applied to the Au electrode, an electric current which results from the oxidization of intercalators is observed. As shown in the inset of Fig. 30.5.2, the magnitude of the electric current differs if the double-stranded DNA forms or not. In turn, the sequence of the target DNA can be discriminated by measuring the magnitude of the electric current on each electrode.

Labeling of target DNA with dyes and with enzymes usually requires an additional manual process that takes 1–2 hours. Thus, elimination of the labeling process in our method leads to a simplification of the operation and a reduction in the detection time. Since costly dye and enzyme are unnecessary, our method also leads to a reduction in chip cost. Thus, our electrochemical DNA chip system is a promising platform for personalized medicine.

So far, a DNA chip has been developed for single nucleotide polymorphism (SNP) detection [4] in which precise DNA identification is indispensable. In order to establish precise SNP analysis, conditions of DNA hybridization were optimized. In parallel with SNP analysis, gene expression analysis is also very important from a medical point of view, because it reflects personal healthy conditions. To realize gene expression analysis, both high sensitivity and wide dynamic range of DNA chip are required.

In the work reported in this paper, a DNA chip has been developed with a CMOS circuit for quantitative analysis. A CMOS-type DNA chip is expected to be highly sensitive and detect much lower molecular concentrations than conventional DNA chips, because it can detect lower electric current. Figure 30.5.2 shows the system architecture of our CMOS-type DNA chip. The DNA sensor is operated by a standard potentiostat configuration. Anodic electric current from the intercalators is measured using cyclic voltammetry with a linear voltage sweep. The potential control unit controls the voltage of a working electrode (WE) and counter electrode (CE) in the DNA sensor. The output electric current is amplified by the current mirror circuit and is converted to voltage by the I-V converter. The photograph of the fabricated CMOS-type DNA chip is shown in Fig. 30.5.3. In this configuration, four DNA sensors (WEs) are located in the center of each cell, and isolated from reference electrode (RE) and CE. In the process of immobilization of probe DNA, solutions of probe DNA can be spotted only on the DNA sensor, not on the CE and RE, which enables us to realize the excellent potentiostat analysis. Figure 30.5.4 shows the specification of the CMOS chip. While power dissipation is rather large, the temperature of the sensor region is raised only slightly because the time required for measuring electric currents is only 3 seconds.

Figure 30.5.5 shows the dependence of the output peak current i_p on the target DNA concentration in the analyte solution, obtained by preliminary experiments utilizing 200μm diameter DNA sensors fabricated on the glass substrate. The concentration range that the sensor can detect is about two orders of magnitude. The upper limit of the detection corresponds to the saturation of the hybridization as shown schematically in the inset of Fig. 30.5.5. The lower limit is limited by the response of the non-specifically bound intercalators. Since the amount of saturated target DNA is determined by the amount of the probe DNA on the sensor, the saturation of the hybridization occurs at lower target DNA concentrations in the case of smaller sized sensors. So, the range of the sensor detection can be shifted to a lower concentration by decreasing the sensor area. In the case of non-CMOS type DNA chip, its low sensitivity limits the measurable concentration of the target DNA. However, the CMOS-type DNA chip can measure such small electric currents that we expand the dynamic range of the system by combining various sized sensors of the CMOS-type DNA chip, even if the dynamic range of single sized sensor is narrow. Sensors of electrodes have been fabricated with diameters of 200μm, 63μm, 20μm, 6.3μm and 2.0μm in the same chip. Each sensor is combined with its dedicated current amplifier whose current gain is set so as to normalize the current intensity by its own sensor size, namely 1/100, 1/10, 1, 10 and 100, respectively. The minimum signal current will be in the order of 10 pA from the 2.0μm sensor. The lowest concentration range in this setup would be 10^6copy/mL, which is comparable to or better than the fluorescent method.

The DNA sequence we used for analysis was the SNP region of MxA and MBL (mannose binding lectin), which are related with interferon efficacy [4]. Figure 30.5.6 shows the observed electric currents for the sensors with diameters from 200μm to 2.0μm. As shown in Fig. 30.5.6, a small electric current of 10pA from the DNA sensor with diameter of 2μm has been observed. Power spectra analysis on the data in Fig. 30.5.5 shows that the circuitry for each sensor achieves at least 40dB of SNR throughout the whole signal process. Figure 30.5.7 shows the experimental results of DNA hybridization. Although we have not obtained the results for the sensor with diameters of 6.3μm, and 2.0μm, we have successfully detected the electric currents corresponding to the DNA hybridization for the sensor with diameters from 200μm to 20μm.

This paper reports the first demonstration of a quantitative DNA chip system based on the electrochemical method. Our system will lead to gene expression analysis with high sensitivity and accuracy. From the medical viewpoint, it is expected to realize a novel health monitoring system capable of detecting initial stages of diseases such as cancer.

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References:
[1] G. Hardiman, "Microarray Platforms – Comparisons and Contrasts", *Pharmacogenomics,* vol. 5, p. 487, 2004
[2] R. Thewes et al., "Sensor Arrays for Fully-Electronic DNA Detection on CMOS," *ISSCC Dig. Tech. Papers*, pp. 350-351, Feb., 2002.
[3] K. Hashimoto et al., "Microfabricated Disposable DNA Sensor for Detection of Hepatitis B Virus DNA", *Sensors and Actuators B*, vol. 46, pp. 220-225, 1998.
[4] M. Takahashi, et al., "Construction of an Electrochemical DNA Chip for Simultaneous Genotyping of Single Nucleotide Polymorphism", *Analyst*, vol. 130, p. 687, 2005.

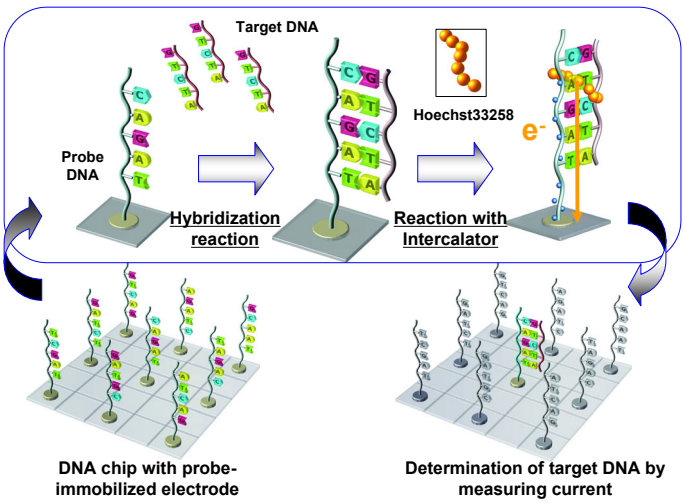


Figure 30.5.1: Principle of Electrochemical DNA Chip.

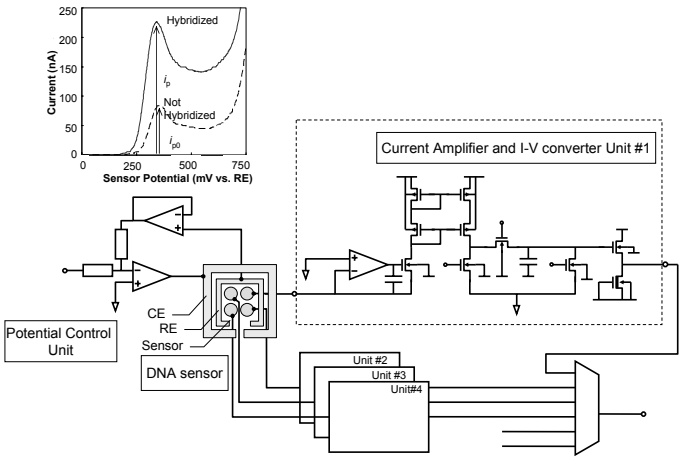


Figure 30.5.2: System Architecture of a CMOS type-DNA Chip.

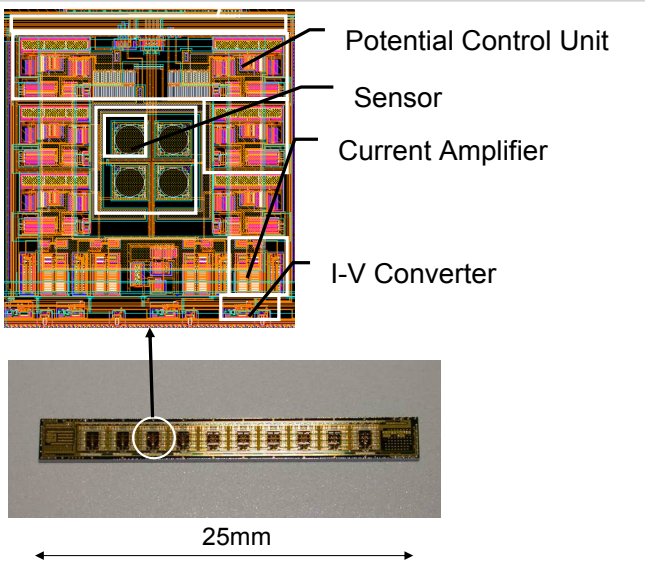


Figure 30.5.3: Micrograph of the fabricated chip.

Technology	CMOS 1μm, Two-Layer Al Interconnection
Supply Voltage	±3.3V
Power Dissipation	150mW
Sensor Diameter	200, 63, 20, 6.3 and 2.0μm
Number of Sensors	8 for Each Size, Total 40
Number of MOSFETs	1600
Chip Die Size	25mm x 3mm

Figure 30.5.4: Chip Performance Summary.

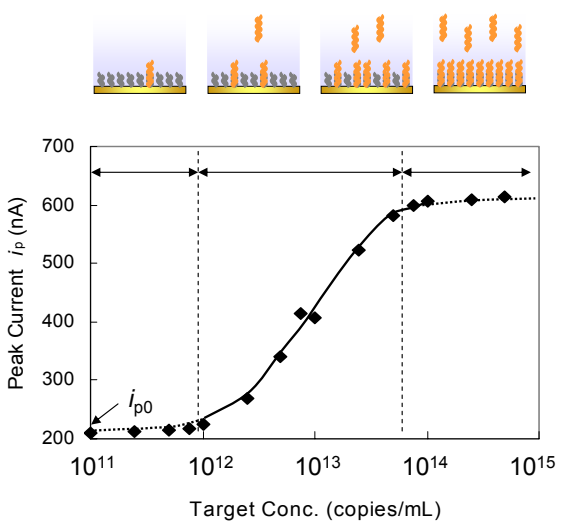
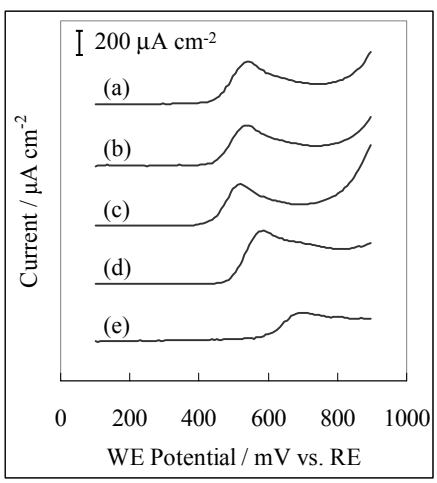


Figure 30.5.5: Dependence of the output peak current i_p on the concentration of target DNA molecule *s*.



(a) ϕ200μm
(b) ϕ 63 μm
(c) ϕ 20 μm
(d) ϕ6.3 μm
(e) ϕ 2 μm

Figure 30.5.6: Measured electric currents for DNA sensors with diameters from 200μm to 2μm.

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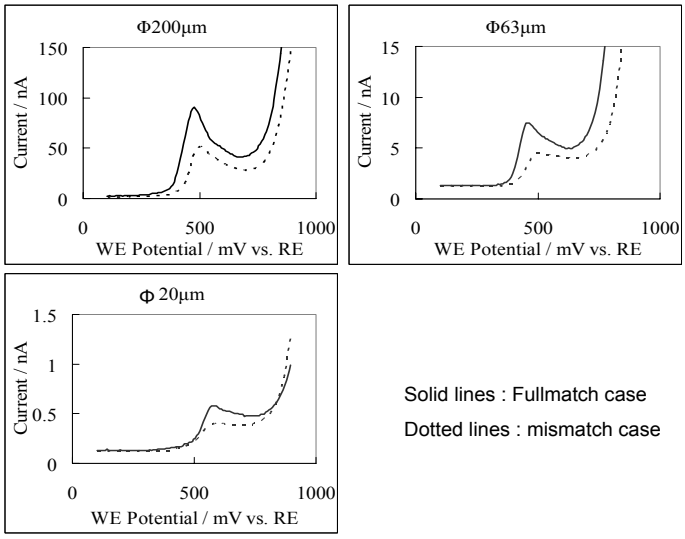

Figure 30.5.7: Measured electric currents corresponding to DNA hybridizations for sensors with diameters from 200μm to 20μm.

